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A novel target to inhibit angiogenesis

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## A novel target to inhibit angiogenesis

### Field of the invention

The invention relates to the field of pathological angiogenesis. In particular the invention relates to the use of molecules binding to AC133 that can be used for the manufacture of a medicament to prevent angiogenesis.

### Background of the invention

The "hemangioblast" is a putative progenitor cell that has the potential to form either endothelial or hematopoietic cells. It exists during embryogenesis in the blood island region of the yolk sac (1), which is therefore the earliest site of hematopoiesis and vasculogenesis. Until recently, vasculogenesis has been thought to be restricted to the yolk sac and the early embryogenesis. However, novel observations have revealed in adulthood a situation consistent with vasculogenesis: endothelial cells derived from angioblasts or "hemangioblasts" previously isolated from peripheral blood or bone marrow are incorporated into sites of neovascularization in physiological and pathological conditions (2-6). In addition, the number of these endothelial cell progenitors increases in the peripheral blood during tissue ischemia or following the administration of VEGF or GM-CSF, a cytokine known to induce mobilization of hematopoietic stem cells from the bone marrow into the peripheral blood (5, 6). Recent studies in humans, dogs, rats, rabbits and mice have indeed indicated the presence of endothelial precursor cells (EPCs) in bone marrow and peripheral blood during adult life which can be mobilized and incorporated into newly formed vessels or are involved in endothelialization of implants (4, 7-13). Interestingly, in all these experiments, EPCs are isolated together with other hematopoietic stem cells by using antibodies directed against hematopoietic stem cell antigens. AC133 is a rather novel human hematopoietic stem cell antigen (14) of unknown physiological or pathological function. AC133, recently designed CD133 (National Center for Biotechnology, 2000) is expressed on lineage non-committed stem and progenitor cells but not on mature peripheral blood cells and umbilical vein derived endothelial cells (15). It is detected on 30-60% of all CD34<sup>+</sup> cells, including CD34<sup>bright</sup> cells. This cell population contains CD38<sup>dim/neg</sup>, HLA-DR<sup>-</sup>, CD117<sup>+</sup>, CD90<sup>+</sup> cells. AC133<sup>+</sup> CD34<sup>+</sup> hematopoietic stem cells

are enriched in Long-Term Culture-Initiating Cells, NOD/SCID repopulating cells and dendritic cell progenitors (16). Subsets of this population express the angiopoietin receptors TIE (67.6%) and TEK (36.8%), Flt-1 (7%), Flt-4 (3.2%), KDR (10.4%), the receptor tyrosine kinase HER-2 (15.4%) and Flt-3 (77.6%). Only few AC133<sup>+</sup> cells do not co-express CD34: these cells are very small and define a population of unknown delineation (CD71<sup>-</sup>, CD117<sup>-</sup>, CD10<sup>-</sup>, CD38<sup>low</sup>, CD135<sup>+</sup>, HLA-DR<sup>high</sup>) (17). In acute myeloid leukemias, AC133 expression is often but not always associated with CD34 expression (18, 19). AC133 is also found on acute lymphoid leukemia blasts and on a subset of CD34<sup>+</sup> B-cell precursors (20). Flow cytometric analyses of a wide panel of human cell lines showed that only retinoblastoma and teratocarcinoma cell lines express AC133 (21). Recently, AC133 was found to be expressed in EPCs. CD34<sup>+</sup> cells co-expressing VEGFR-2 and AC133 have been isolated from peripheral blood, cord blood, fetal liver and bone marrow. However, the possible role of AC 133 in hematopoiesis and vasculogenesis in the developing embryo and, after birth, in angiogenesis, postnatal vasculogenesis and hematopoietic stem cell trafficking, remains largely unknown. To study in detail the *in vivo* role of AC133 in the present invention AC133 deficient mice were generated. It was surprisingly found that AC 133 has a key role in pathological vasculogenesis and/or angiogenesis and that inhibitors of AC133 can be used in therapeutic strategies to inhibit blood vessel formation in various pathological disorders.

#### Aims and detailed description of the invention

AC133 is a protein of 97 KD with a 5-transmembrane structure (14). The 5-transmembrane structure appears at present restricted to the human AC133, the murine prominin and the related protein of the nematode *C. elegans* (25-28). This structure indicates that these proteins belong to a new class of serpentine receptors. Murine prominin has 60% amino acid homology with AC133 (25). It is not yet clear whether murine prominin is the murine homologue of human AC133, or whether it is a close family member (25, 26). For the sake of clarity the nucleotide sequence of human AC133 is designated here as SEQ ID NO: 1 and the amino acid sequence of human AC133 is designated as SEQ ID NO: 2. The present invention shows that inhibitors of AC133 can be used in therapeutic applications for the prevention of pathological angiogenesis.

Thus the invention provides in one embodiment the use of a molecule which comprises a region specifically binding to AC133 or nucleic acids encoding AC133, for the manufacture of a medicament to treat pathological angiogenesis.

- 5 According to the invention molecules that comprise a region specifically binding to AC133 or nucleic acids encoding AC133 which can be used for the manufacture of a medicament to treat pathological angiogenesis can be chosen from the list comprising an antibody or any fragment thereof binding to AC133, a (synthetic) peptide, a protein, a small molecule specifically binding to AC133 or nucleic acids encoding AC133, a  
10 ribozyme against nucleic acids encoding AC133, and anti-sense nucleic acids hybridising with nucleic acids encoding AC133.

- The term 'antibody' or 'antibodies' relates to an antibody characterized as being specifically directed against AC133 or any functional derivative thereof, with said  
15 antibodies being preferably monoclonal antibodies; or an antigen-binding fragment thereof, of the F(ab')<sub>2</sub>, F(ab) or single chain Fv type, or any type of recombinant antibody derived thereof. These antibodies of the invention, including specific polyclonal antisera prepared against AC133 or any functional derivative thereof, have no cross-reactivity to others proteins. The monoclonal antibodies of the invention can  
20 for instance be produced by any hybridoma liable to be formed according to classical methods from splenic cells of an animal, particularly of a mouse or rat immunized against AC133 or any functional derivative thereof, and of cells of a myeloma cell line, and to be selected by the ability of the hybridoma to produce the monoclonal antibodies recognizing AC133 or any functional derivative thereof which have been  
25 Initially used for the immunization of the animals. The monoclonal antibodies according to this embodiment of the invention may be humanized versions of the mouse monoclonal antibodies made by means of recombinant DNA technology, departing from the mouse and/or human genomic DNA sequences coding for H and L chains or from cDNA clones coding for H and L chains. Alternatively the monoclonal  
30 antibodies according to this embodiment of the invention may be human monoclonal antibodies. Such human monoclonal antibodies are prepared, for instance, by means of human peripheral blood lymphocytes (PBL) repopulation of severe combined immune deficiency (SCID) mice as described in PCT/EP 99/03605 or by using transgenic non-human animals capable of producing human antibodies as described

in US patent 5,545,806. Also fragments derived from these monoclonal antibodies such as Fab, F(ab)<sub>2</sub> and ssFv ("single chain variable fragment"), providing they have retained the original binding properties, form part of the present invention. Such fragments are commonly generated by, for instance, enzymatic digestion of the antibodies with papain, pepsin, or other proteases. It is well known to the person skilled in the art that monoclonal antibodies, or fragments thereof, can be modified for various uses. The antibodies involved in the invention can be labeled by an appropriate label of the enzymatic, fluorescent, or radioactive type.

In a specific embodiment the antibodies against AC133 can be derived from animals of the camelid family. In said family immunoglobulins devoid of light polypeptide chains are found. Heavy chain variable domain sequences derived from camelids are designated as VHH's. "Camelids" comprise old world camelids (*Camelus bactrianus* and *Camelus dromaderius*) and new world camelids (for example *Lama paccos*, *Lama glama* and *Lama vicugna*). EP0656946 describes the isolation and uses of camelid immunoglobulins and is incorporated herein by reference.

Small molecules, e.g. small organic molecules, and other drug candidates can be obtained, for example, from combinatorial and natural product libraries.

Also within the scope of the invention are oligoribonucleotide sequences, that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of AC133 mRNA. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between -10 and +10 regions of the AC133 nucleotide sequence, are preferred. Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of AC133 RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of

between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize anti-sense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

The term 'pathological angiogenesis' refers to the formation and growth of blood vessels during the maintenance and the progression of several disease states. Examples where pathological angiogenesis can occur are blood vessels (atherosclerosis, hemangioma, hemangioendothelioma), bone and joints (rheumatoid arthritis, synovitis, bone and cartilage destruction, osteomyelitis, pannus growth, osteophyte formation, neoplasms and metastasis), skin (warts, pyogenic granulomas, hair growth, Kaposi's sarcoma, scar keloids, allergic oedema, neoplasms), liver, kidney, lung, ear and other epithelia (inflammatory and infectious processes (including hepatitis, glomerulonephritis, pneumonia), asthma, nasal polyps, otitis, transplantation, liver regeneration, neoplasms and metastasis), uterus, ovary and placenta (dysfunctional uterine bleeding (due to intrauterine contraceptive devices), follicular cyst formation, ovarian hyperstimulation syndrome, endometriosis, neoplasms), brain, nerves and eye (retinopathy of prematurity, diabetic retinopathy, choroidal and other intraocular disorders, leukomalacia, neoplasms and metastasis), heart and skeletal muscle due to work overload, adipose tissue (obesity), endocrine organs (thyroiditis, thyroid enlargement, pancreas transplantation), hematopoiesis (AIDS (Kaposi),

hematologic malignancies (leukemias, etc.), lymph vessels (tumour metastasis, lymphoproliferative disorders).

The term 'medicament to treat' relates to a composition comprising molecules as described above and a pharmaceutically acceptable carrier or excipient (both terms can be used interchangeably) to treat diseases as indicated above. Suitable carriers or excipients known to the skilled man are saline, Ringer's solution, dextrose solution, Hank's solution, fixed oils, ethyl oleate, 5% dextrose in saline, substances that enhance isotonicity and chemical stability, buffers and preservatives. Other suitable carriers include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids and amino acid copolymers. The 'medicament' may be administered by any suitable method within the knowledge of the skilled man. The preferred route of administration is parenterally. In parental administration, the medicament of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with the pharmaceutically acceptable excipients as defined above. However, the dosage and mode of administration will depend on the individual. Generally, the medicament is administered so that the protein, polypeptide, peptide of the present invention is given at a dose between 1  $\mu\text{g/kg}$  and 10  $\text{mg/kg}$ , more preferably between 10  $\mu\text{g/kg}$  and 5  $\text{mg/kg}$ , most preferably between 0.1 and 2  $\text{mg/kg}$ . Preferably, it is given as a bolus dose. Continuous infusion may also be used and includes continuous subcutaneous delivery via an osmotic minipump. If so, the medicament may be infused at a dose between 5 and 20  $\mu\text{g/kg/minute}$ , more preferably between 7 and 15  $\mu\text{g/kg/minute}$ .

In another embodiment antibodies or functional fragments thereof can be used for the manufacture of a medicament for the treatment of the above mentioned disorders. As a non-limiting example there are the antibodies described in US 5,843,633. In a specific embodiment said antibodies are humanized (Rader et al., 2000, J. Biol. Chem. 275, 13688) and more specifically human antibodies are used to manufacture a medicament to treat pathological angiogenesis. In yet another specific embodiment antibodies derived from camelids are used to manufacture a medicament to treat pathological angiogenesis.



Another aspect of administration for treatment is the use of gene therapy to deliver the above mentioned anti-sense gene or functional parts of the AC133 gene or a ribozyme directed against the AC133 mRNA or a functional part thereof. Gene therapy means the treatment by the delivery of therapeutic nucleic acids to patient's cells. This is extensively reviewed in Lever and Goodfellow 1995; Br. Med Bull.,51, 1-242; Culver 1995; Ledley, F.D. 1995. Hum. Gene Ther. 6, 1129. To achieve gene therapy there must be a method of delivering genes to the patient's cells and additional methods to ensure the effective production of any therapeutic genes. There are two general approaches to achieve gene delivery; these are non-viral delivery and virus-mediated gene delivery.

The invention also provides methods for identifying compounds or molecules which bind on AC133 and prevent or suppress pathological angiogenesis. With "suppression" it is understood that said suppression can occur for at least 20%, 30%, 30%, 50%, 60%, 70%, 80%, 90% or even 100%.

Thus in another embodiment the invention provides a method to identify molecules that comprise a region that specifically binds to AC133 comprising: (1) exposing AC133 or nucleic acids encoding AC133 to at least one molecule whose ability to suppress or prevent pathological angiogenesis is sought to be determined, (2) determining binding or hybridising of said molecule(s) to AC133 or nucleic acids encoding AC133, and (3) monitoring said pathological angiogenesis when administering said molecules as a medicament.

The latter method is also referred to as 'drug screening assay' or 'bioassay' and typically include the step of screening a candidate/test compound or agent for the ability to interact with AC133. Candidate compounds or agents, which have this ability, can be used as drugs to combat or prevent pathological conditions of angiogenesis. Candidate/test compounds such as small molecules, e.g. small organic molecules, and other drug candidates can be obtained, for example, from combinatorial and natural product libraries as described above.

Typically, the assays are cell-free assays which include the steps of combining AC133 and a candidate/test compound, e.g., under conditions which allow for interaction of (e.g. binding of) the candidate/test compound with AC133 to form a complex, and detecting the formation of a complex, in which the ability of the candidate compound to

interact with AC133 is indicated by the presence of the candidate compound in the complex. Formation of complexes between AC133 and the candidate compound can be quantitated, for example, using standard immunoassays. The AC133 employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located extracellularly or even intracellularly.

To perform the above described drug screening assays, it is feasible to immobilize AC133 or its (their) target molecule(s) to facilitate separation of complexes from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Interaction (e.g., binding of) of AC133 to a target molecule, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and microcentrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows the protein to be bound to a matrix. For example, AC133-His tagged can be adsorbed onto Ni-NTA microtitre plates, or AC133-ProtA fusions adsorbed to IgG, which are then combined with the cell lysates (e.g., <sup>35</sup>S-labeled) and the candidate compound, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the plates are washed to remove any unbound label, and the matrix immobilized and radiolabel determined directly, or in the supernatant after the complexes are dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of AC133-binding protein found in the bead fraction quantitated from the gel using standard electrophoretic techniques. Other techniques for immobilizing protein on matrices can also be used in the drug screening assays of the invention. For example, AC133 can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated AC133 can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Another technique for drug screening which provides for high throughput screening of compounds having suitable binding affinity to AC133 is described in detail in "Determination of Amino Acid Sequence Antigenicity" by Geysen HN, WO 84/03564, published on 13/09/84. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The protein test compounds are reacted with fragments of AC133 and washed. Bound AC133 is then detected by methods well known in the art. Purified

AC133 can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support. This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding AC133 specifically compete with a test compound for binding AC133. In this manner, the antibodies can be used to detect the presence of any protein, which shares one or more antigenic determinants with AC133.

### Examples

#### 1. Generation of a AC133 knock-out mouse

Targeted inactivation of the AC133 gene was achieved by deletion of exon 2 (containing the start codon). Briefly, a genomic BAC (bacterial artificial chromosome) containing the murine AC133 (14) was obtained from Research Genetics Inc (Huntsville, AL) after screening by PCR and hybridization. Mapping of the murine AC133 homologue gene revealed that the first exon, which is 79 bp long, is separated from the second exon by an approximately 8 kb intron. It is the second exon (376 bp long) which contains the startcodon ATG. A BamHI fragment of 11.5 kb containing exon 2 was subcloned into the pUC18 plasmid. A targeting vector for inactivation of the AC133 gene, *pPNT.AC133<sup>null</sup>*, was constructed consisting of, from 5' to 3': 1.2 kb of 5' homology comprising the end of intron 1; a *loxP*-flanked *neomycin* gene; 5.5 kb from intron 2 as 3'-homology; and a *thymidine kinase* selection cassette outside of the regions of homology for counterselection against random integration events. The integrity of the construct was verified by restriction digestion and sequencing. The linearized targeting vector *pPNT.AC133<sup>null</sup>* was electroporated in R1 ES cells and targeted clones were identified by appropriate Southern blot analysis and used for morula aggregation to generate AC133 deficient chimeric and germline mice. AC133<sup>-/-</sup> mice were born at the expected Mendelian frequency (~25% of 450 offspring from AC133<sup>+/-</sup> breeding pairs). They appeared healthy and were fertile, irrespective of their genetic background (backgrounds tested: 50% Swiss/50% 129, 100% 129, 50% C57Bl6/50% 129 and 50% NMRI/50% 129). We anticipated that AC133 might play a crucial role in hematopoiesis implying that the AC133<sup>-/-</sup> embryo would die *in utero* either after the appearance of the primitive hematopoiesis (7.5 days post coitum, site:

yolk sac) or at the emergence of the definitive hematopoiesis (12.5 days post coitum, site: fetal liver). Surprisingly, however, embryonic development in AC133<sup>-/-</sup> mice was normal. AC133<sup>-/-</sup> embryos were not rescued by maternal AC133, as AC133<sup>-/-</sup> embryos, sired by AC133<sup>+/-</sup> as well as by AC133<sup>-/-</sup> breeding pairs, developed normally. Also postnatal physiological vascular development seemed normal since no vascular defects could be observed in the heart, kidneys, lungs and skeletal muscle during postnatal growth in AC133<sup>-/-</sup> mice.

## 2. Impaired pathological angiogenesis in AC133 knock-out mice

In order to study the possible role of AC133 in pathological conditions of angiogenesis AC 133<sup>-/-</sup> mice and their wild-type littermates were subjected to a mouse model of ischemic retinopathy. In this hyperoxia-induced retinopathy model, neonatal mice (with an immature retinal vasculature) are exposed to hyperoxia, resulting in obliteration of the developing blood vessels supplying oxygen to the retina. When the mice are then returned to normoxia, the retina, distal to the occluded vessels, becomes ischemic, inducing VEGF production and ultimately resulting in reproducible and quantifiable proliferative retinal neovascularization (29, 30). This model, which mimicks to a certain extent the vascular response during retinopathy of prematurity or diabetic retinopathy, may be useful to test the efficacy of (anti)-angiogenic molecules (31). Mouse pups of 7 days (P7) together with their mothers, are subjected to hyperoxia (75% oxygen) in specially designed oxygen chambers for 5 days, without opening the cages. On P12, the animals are returned to room air until P17, when the retinas are assessed for maximal neovascular response. Anesthetized mice are perfused through the left ventricle with 1 ml of phosphate buffered saline containing 50 mg of 2x10<sup>6</sup> molecular weight fluorescein-dextran. The eyes are removed and fixed in 4% paraformaldehyde for 3 (right eye) or 24 (left eye) hrs. Of the right eyes, lenses are removed and peripheral retinas cut to allow flat mounting with glycerol-gelatin. The flat mounted retinas are analyzed by fluorescence microscopy. The left eyes are embedded in paraffin and serial 6 µm sections are cut sagittally throughout the cornea, parallel to the optic nerve, and stained with hematoxylin-eosin. The proliferative neovascular response is quantified by counting the number of new vessels (= tufts) and the number of endothelial cells extending from the internal limiting membrane of the retina into the vitreum on the stained sagittal cross-sections. The angiographic technique using

fluorescein-dextran perfusion is used in conjunction with this counting method for rapid screening of retinas or as an alternative grading system for quantitative evaluation.

Loss of AC133 significantly protected mice against intra-vitreous neovascularization, as evaluated by counting the number of neovascular tufts and endothelial cells (EC) in the vitreous cavity (n=15; p<0.001)

	N° of tufts in vitreous cavity (per 10 retinal sections)	N° of EC in vitreous cavity (per 10 retinal sections)
AC133 <sup>+/+</sup> pups (n = 15)	157.1 ± 13.6	286.0 ± 45.1
AC133 <sup>-/-</sup> pups (n = 15)	72.5 ± 14.6	106.2 ± 22.6

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12. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001;89(1):E1-7.
13. Gill M, Dias S, Hattori K, Rivera ML, Hicklin D, Witte L, et al. Vascular trauma induces rapid but transient mobilization of VEGFR2(+)AC133(+) endothelial precursor cells. *Circ Res* 2001;88(2):167-74.
14. Miraglia S, Godfrey W, Yin AH, Atkins K, Warnke R, Holden JT, et al. A novel five-transmembrane hematopoietic stem cell antigen: Isolation, characterization, and molecular cloning. *Blood* 1997;90(12):5013-21.
15. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 1997;90(12):5002-12.
16. de Wynter EA, Buck D, Hart C, Heywood R, Coutinho LH, Clayton A, et al. CD34+AC133+ cells isolated from cord blood are highly enriched in long-term culture-initiating cells, NOD/SCID-repopulating cells and dendritic cell progenitors. *Stem Cells* 1998;16(6):387-96.
17. Buhring HJ, Seiffert M, Bock TA, Scheduling S, Thiel A, Scheffold A, et al. Expression of novel surface antigens on early hematopoietic cells. *Ann N Y Acad Sci* 1999;872:25-38; discussion 38-9.
18. Horn PA, Tesch H, Staib P, Kube D, Diehl V, Voliotis D. Expression of AC133, a novel hematopoietic precursor antigen, on acute myeloid leukemia cells. *Blood* 1999;93(4):1435-7.
19. Kratz-Albers K, Zuhlsdorf M, Leo R, Berdel WL, Buchner T, Serve H. Expression of a AC133, a novel stem cell marker, on human leukemic blasts lacking CD34-antigen and on a human CD34+ leukemic line:MUTZ-2. *Blood* 1998;92(11):4485-7.
20. Buhring HJ, Seiffert M, Marxer A, Weiss B, Faul C, Kanz L, et al. AC133 antigen expression is not restricted to acute myeloid leukemia blasts but is also found on acute lymphoid leukemia blasts and on a subset of CD34+ B-cell precursors. *Blood* 1999;94(2):832-3.
21. Corbeil D, Roper K, Hannah MJ, Hellwig A, Huttner WB. Selective localization of the polytopic membrane protein prominin in microvilli of epithelial cells - a combination of apical sorting and retention in plasma membrane protrusions. *J Cell Sci* 1999;112(Pt 7):1023-33.

22. Gehling UM, Ergul S, Schumacher U, Wagener C, Pantel K, Otte M, et al. In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 2000;95(10):3106-12.
23. Peichev M, Naylor AJ, Pereira D, Zhu Z, Lane WJ, Williams M, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 2000;95(3):952-8.
24. Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL. Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. *Br J Haematol* 2001;115(1):186-94.
25. Corbell D, Roper K, Weigmann A, Huttner WB. AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? *Blood* 1998;91(7):2625-6.
26. Miraglia S, Godfrey W, Buck D. A response to AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? *Blood* 1998;91(11):4390-1.
27. Wilson R, Ainscough R, Anderson K, Baynes C, Berks M, Bonfield J, et al. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. *Nature* 1994;368(6466):32-8.
28. Weigmann A, Corbell D, Hellwig A, Huttner WB. Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. *Proc Natl Acad Sci U S A* 1997;94(23):12425-30.
29. Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'Amato R, Sullivan R, et al. Oxygen-induced retinopathy in the mouse. *Invest Ophthalmol Vis Sci* 1994;35(1):101-11.
30. Wesolowski E, Smith LE. Effect of light on oxygen-induced retinopathy in the mouse. *Invest Ophthalmol Vis Sci* 1994;35(1):112-9.
31. Pierce EA, Avery RL, Foley ED, Aiello LP, Smith LE. Vascular endothelial growth factor/vascular permeability factor expression in a mouse model of retinal neovascularization. *Proc Natl Acad Sci U S A* 1995;92(3):905-9.



Claims

1. Use of a molecule which comprises a region specifically binding to AC133 or nucleic acids encoding AC133, for the manufacture of a medicament to treat pathological angiogenesis.

2. Use according to claim 1 wherein said molecule is chosen from the list comprising an antibody or any fragment thereof binding to AC133, a small molecule specifically binding to AC133 or nucleic acids encoding AC133, a ribozyme against nucleic acids encoding AC133, and anti-sense nucleic acids hybridising with nucleic acids encoding AC133.

3. A method to identify molecules that comprise a region that specifically binds to AC133 comprising:

- exposing AC133 or nucleic acids encoding AC133 to at least one molecule whose ability to suppress or prevent pathological angiogenesis is sought to be determined,
- determining binding or hybridising of said molecule(s) to AC133 or nucleic acids encoding AC133, and
- monitoring said pathological angiogenesis when administering said molecules as a medicament.

### Abstract

The invention relates to the field of pathological angiogenesis. In particular the invention relates to the use of molecules binding to AC133 that can be used for the manufacture of a medicament to prevent angiogenesis.

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 Val Leu Asn Ser Ile Gly Ser Asp Ile Asp Asn Val Thr Gln Arg Leu  
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 Pro Ile Gln Asp Ile Leu Ser Ala Phe Ser Val Tyr Val Asn Asn Thr  
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 Glu Ser Tyr Ile His Arg Asn Leu Pro Thr Leu Glu Glu Tyr Asp Ser  
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 Asp Thr Pro Tyr Leu Leu Asn Glu Asp Trp Glu Tyr Tyr Leu Ser Gly  
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His  
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